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Fiber optic SPR biosensing platform for multiplex DNA quantification and identification

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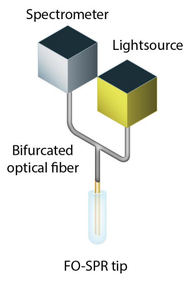
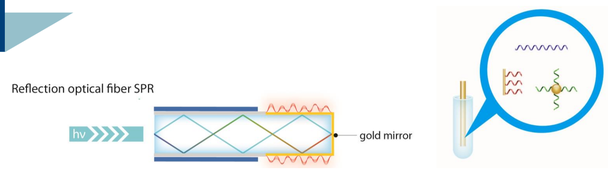
INTRODUCTION

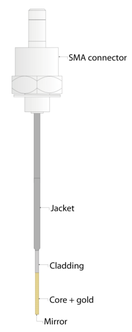
- Problem statement
  - **Accurate identification** and **quantification** of bacteria and viruses is key in food quality, healthcare and biotechnology
  - Golden standard: (q)PCR followed by HRM (**end-point method**)
  - Degree of multiplexing limited in qPCR and HRM due to **spectral overlap** between emission spectra of expensive fluorescent reporters
  - qPCR largely incompatible with the concept of **point-of-care** (POC)
- Objectives
  - **Real-time simultaneous detection** of two related bacteria
  - **Quantification** of different micro-organisms
  - **Discrimination** of mutations
  - Miniaturization possibilities towards **POC diagnostics**



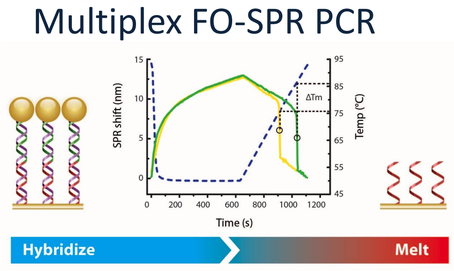
METHODS

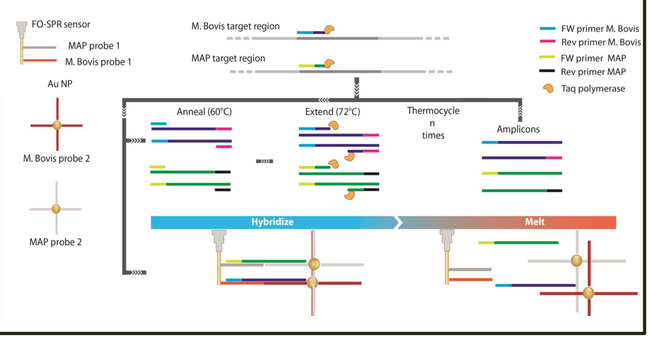
- FO SPR





- Bacteria
  - *Mycobacterium avium paratuberculosis* (MAP), 56 bp
  - *Mycobacterium bovis* (M. Bovis), 76 bp
- Melting analysis [1-6]
- Multiplex FO-SPR PCR

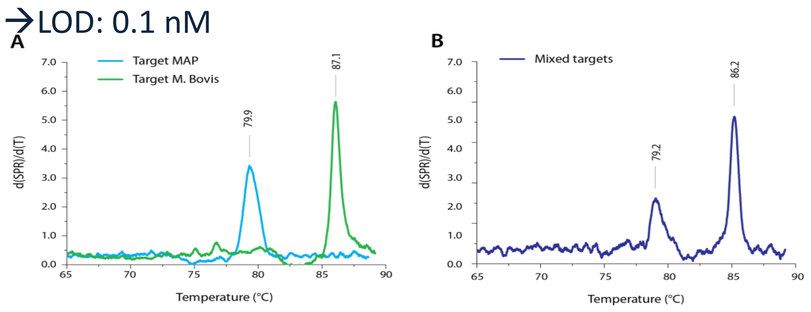




RESULTS

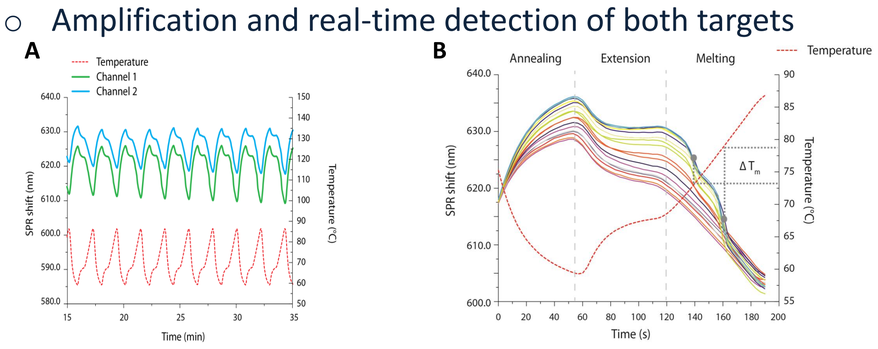
- Multiplex FO-SPR melting assay (dS/dT)
  - Both targets can be identified simultaneous

→LOD: 0.1 nM



A) Individual FO-SPR melting analysis for the MAP (500 nM) and M. Bovis target (500 nM) sequence.
B) Multiplex FO-SPR melting analysis for the MAP and M. Bovis target sequence, 250 nM each.

- Multiplex FO-SPR PCR assay
  - Amplification and real-time detection of both targets



A) FO-SPR signal: raw data for 9 cycles in a multiplex PCR reaction containing bacterial DNA of both MAP and M. Bovis at a concentration of 1 nM.
B) FO-SPR signal for each PCR cycle Initially the FO-SPR signal is the exact inverse of the temperature signal, however as the DNA reaches the detection limit of the FO-SPR sensor, a melting signal for each DNA target is visible.

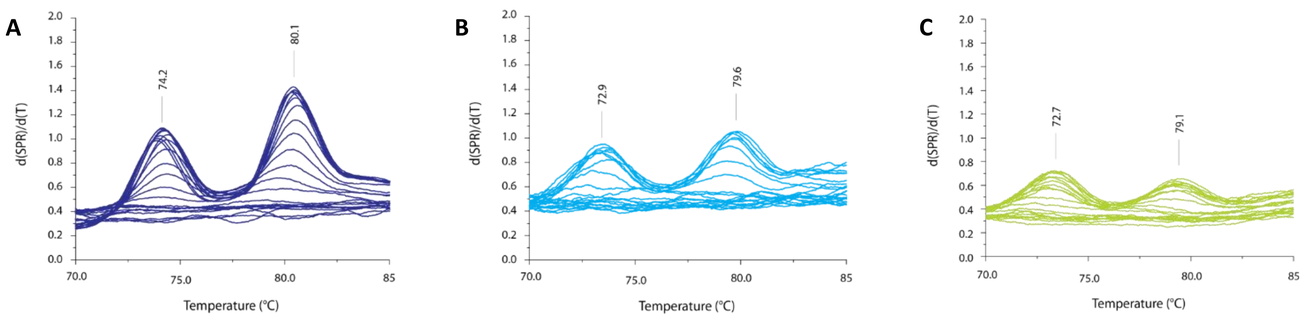
- Processing FO-SPR PCR assay data (dS/dT)
  - Easy to identify both targets
  - Very precise determination of T_m

→ ΔT_m: 5.7 °C

First order derivative of the FO-SPR signal and temperature, which allows to resolve the melting point of the two target DNA types amplified with the multiplex PCR. The MAP sequence has a lower melting point, because it is considerably shorter while the M. Bovis target has a higher T_m. Both targets can easily be resolved as the signals are separated by 5.7 °C.

- Real-time FO-SPR mutation analysis (dS/dT)
  - T_m of shorter target more influenced by SNP
  - Signal height of longer target more influenced by SNP

→ Discrimination of wild type, single SNP and three SNPs



A) FO-SPR PCR analysis of the wild type MAP and M. Bovis target sequence at a concentration of 10 pM. B) FO-SPR PCR melt analysis of MAP and M. Bovis sequences bearing a single SNP (MM1). C) FO-SPR PCR melt analysis of MAP and M. Bovis sequences bearing three SNPs (MM3).

CONCLUSION

- FO-SPR melting assay of DNA
  - End-point method
  - Differentiation of 2 bacterial targets
  - Compared to HRM:
    - 1000 fold increase in DNA LOD
    - 10 fold increase in discriminatory power
- PCR assay on a single optical fiber
  - Targets discriminated by melting profile: proof of concept
  - Multiplex amplification
  - Real-time detection of 2 related bacteria
  - SNP sensitive quantification

FOX DIAGNOSTICS

REFERENCES & ACKNOWLEDGEMENTS

Faculty of Bioscience engineering, Department of Biosystems, MeBioS Biosensor group, Willem de Croylaan 42, B-3001 Heverlee
[1]*Small*, 2011, **7**, 1003; [2]*Nanotechnology*, 2012, **23**, 065503; [3]*Small*, 2012, **8**, 868; [4]*Sensors*, 2013, **1**, 1353;
[5]*Analytical chemistry*, 2013, **3**, 1734; [6]*Nanotechnology*, 2012, **23**, 235503;